# A BLACK KNOT DISEASE OF DIANTHERA AMERICANA L.

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(WITH PLATES 58-61, CONTAINING 10 FIGURES)

#### Introduction

During the past two years the water willow, Dianthera americana, growing along a small creek near the campus of the University of Texas has been affected with a disease which does not appear to have been heretofore reported. Affected plants were first observed during the summer of 1910, but owing to the pressure of other duties at that time the investigation was only superficial and failed to reveal the true nature of the trouble. At the beginning of the present season however, it was made a subject of special investigation which has brought out clearly the nature of the disease and all of the salient features in the life history of the causal organism. A few points which as yet are not fully determined will be more carefully followed this coming season.

### SYMPTOMS OF THE DISEASE

The disease affects the aerial portions of the plant and produces numerous hypertrophied areas of the internodes. These areas are not localized on any particular portion of the stem but occur at irregular intervals beginning near the base and extending to the tip. The internode which bears the inflorescence, and midrib of the leaf are frequently affected. The swollen areas vary in length from one to three centimeters and usually completely encircle the stem. The hypertrophy is not uniform but occurs as distinct ridges opposite the peripheral steles, which in this species of *Dianthera* are six in number (Plate LIX, fig. 1).

As the disease develops the outer tissue of the stem is ruptured by a longitudinal fissure and exposes the developing fungus which presents a smooth grayish surface over a dark background of compact tissue. In typical cases there are six such ruptures, one for each of the peripheral steles. In older stages the fungus areas become somewhat confluent, but they always remain more or less separate. The surface becomes distinctly roughened and papillate with age. The grayish color disappears and the entire area becomes jet black (Plate LVIII, figs. 1 and 2).

#### ETIOLOGY AND EFFECT ON THE HOST

In order to facilitate the study of the relationship of fungus and host, sections were prepared from normal portions of the stem and from affected areas in different stages of development. The material was fixed in chrom-acetic acid fixing solution and imbedded in celloidin. Both longitudinal and transverse sections were prepared and then stained with aniline safranin and Delafield's haematoxylin.

A section through one of the affected areas reveals the fact that the fungus bears a close relation to the vascular tissue of the host, and that certain definite structural modifications are caused by it.

The stem is polystelic, there being seven steles, six of which are disposed in a circle in the peripheral portion while one occupies a position near the center of the stem. The ground tissue is made up of thin-walled parenchymatous cells with large intercellular spaces typical of aquatic or semiaquatic plants. The steles are orbicular in cross section and each is surrounded by a thin-walled, completely closed endodermis. Inside the endodermis there is a layer of thin-walled stereomatic tissue. The mestome bundles are collateral and arranged in an arch toward the periphery of the stem, while the inner face of the stele is occupied by a pith and a few scattered strands of pure leptome. The cambium lies inside the leptome.

Sections taken from portions of the stem somewhat removed from one of the affected areas show the same structure as a normal unaffected stem except that the vessels of the xylem contain numerous fungal filaments (Plate LXI, fig. 3; Pl. LX, fig. 1). In some cases the vessels are completely filled with the filaments of the fungus.

All portions of the affected plants reveal the presence of these

filaments in the vascular tissue but they never invade the ground tissue except in the swollen areas noted above. Sections have been taken from the aerial portions, the rhizome, and the roots. At this season of the year (November) underground portions of the plant are abundantly supplied with the fungal filaments while the aerial parts have died down and almost completely disappeared. It seems highly probable that these filaments persist throughout the winter and begin growth with the aerial portions in the spring. Strength is afforded this hypothesis by the fact that the disease occurs in localized areas while plants somewhat removed are often unaffected. This point however has not been definitely determined.

The fungus causes decided structural changes in the steles and in the ground tissue immediately surrounding them. These changes are affected only in portions of the stem which become hypertrophied as noted above. The steles are generally changed in outline and frequently become branched. The cambium of the inner face is stimulated to produce new xylem cells and frequently a wedge-shaped area results which is greater in extent than the original stele. The cells of this enlarged portion always contain filaments of the fungus (Plate LX, fig. 1).

The loose, lace-like ground tissue surrounding the stele is replaced by a dark, compact parenchyma with no intercellular spaces. This tissue seems to be made up of cells of both fungus and host but in some cases the host tissue is changed beyond the border of the fungus invasion. This parenchyma develops from the side of the stele directed toward the periphery of the stem, while there is little or none of it produced toward the center. The central stele is also usually affected (Plate LIX, fig. 1). Compare cells of the normal ground tissue in parts of Plate LIX with Plate LX, fig. 1.

The fungus, after it reaches the outer part of the stem, forms a layer of rather loose pseudoparenchyma which bursts open the epidermis producing the pulvinate effect already noted. From this tissue the conidiophores arise. The conidiophore layer is very compact in structure and its outer surface is quite smooth and even. The conidiophores produce numerous crops of spores. In cross section this layer is marked by several

concentric lines (Plate LIX, fig. 2). These lines serve to indicate the number of crops of spores produced as they are formed by the broken stubs or remnants of branches from which the spores have fallen. Figure 2, Plate LIX, shows an area which has borne six or seven crops of spores. This figure shows also the shape and outline of the conidiophore layer.

The conidiophores are somewhat branched, septate, packed very closely together and bear spores at the tip and from very short lateral outgrowths near the tip. They continue growth in length by a lateral branch after the spores have fallen and the broken stubs appear in cross section as distinct lines. The conidiospores are unicellular, oval, hyaline and measure 10 to 15 by  $3\mu$  (Plate LXI, fig. 2).

With age this layer begins to slough away, giving the outer surface a very rough ragged appearance. While the conidiophore layer is breaking down a differentiation takes place in the deeper stromatic mass upon which it rests. In transverse sections small cavities appear in the stroma. These are the beginnings of the perithecia and by the time the outer layer of conidiophores have disappeared they are almost fully developed. The perithecia are numerous and closely packed together in the stroma. They are somewhat elongated 475 to 550 by 300 to 350 $\mu$  and produce rather long necks which open by a definite ostiole. The broken remains of the conidiophore layer together with the necks of the perithecia cause the ragged papillate character of the surface as noted above.

The asci are small, 50 to 65 by 10 to  $15\mu$ , thin-walled, and spring from the bottom and sides of the perithecium. The spores are eight in number, biseriate, unicellular, hyaline, allantoid, and measure 6 to 9 by  $2\mu$ . There are no paraphyses (Plate LXI, fig. 4). The perithecia do not develop definite walls but each represents rather a loculus in the stroma (Plate LX, fig. 2).

The cultural characters of the fungus are not at present known, as all attempts to grow it in cultures have failed. Attempts were made to isolate from both the conidiospores and the ascospores as well as the tissue from the stroma but without success.

#### TECHNICAL DESCRIPTION

There does not appear to be any described genus to which this fungus can be unreservedly referred. The structure of the stroma and of the perithecium places it among the Dothidiaceae. In many respects it resembles *Plowrightia*, and were it not for the fact that the spores are unicellular there would be little objection to assigning it to that genus. However, the spores described above are of undoubted maturity and such a classification is therefore untenable.

It appears to more nearly agree with *Bagniesiella* than any other described genus and probably does not differ from it sufficiently to warrant the founding of a new genus. The shape of the spores is perhaps the most important feature which differs. In *B. australis* the spores are elliptical with obtuse ends and subinequilateral and are therefore not markedly different from the spores herein described.

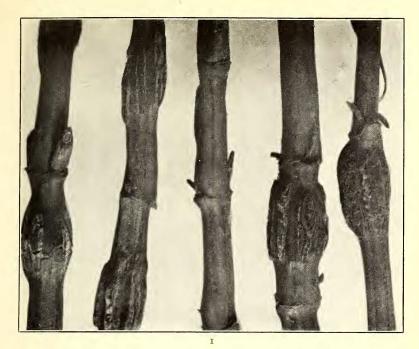
## Bagniesiella Diantherae sp. nov.

Stroma erumpent, pulvinate, linear, 10 to 30 mm. in length by 2 to 4 mm. in diameter, black, smooth at first, becoming roughened and tuberculate with age. Conidial stage appearing before the ascigerous and borne on the same stroma. Conidiophores branched, packed closely together, conidiospores hyaline, oval, unicellular,  $10-15 \times 3\mu$ . Perithecia numerous, subglobose to elongate, immersed in the stroma,  $475-550 \times 300-350\mu$ . Necks elongate, ostiolate. Asci clavate,  $50-65\mu \times 10-15\mu$ , without paraphyses, 8-spored. Ascospores biseriate, hyaline, continuous allantoid,  $6-9 \times 2\mu$ .

On living stems of Dianthera americana at Austin, Texas.

In conclusion, the writer wishes to acknowledge his indebtedness to Mrs. Flora W. Patterson for her opinion as to the relationship of the fungus.

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BAGNIESIELLA DIANTHERAE LEWIS